Supporting Information

Quantitatively Resolving Ligand-Receptor Bonds on Cell Surfaces Using Force-Induced Remnant Magnetization Spectroscopy

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1. Control Experiments for the Substrate Surface

The results in Figure S1 confirm that the magnetic signal decrease in the 60-100 pN region in Figure 3a belong to the dissociation of the specific CD4 bonds.

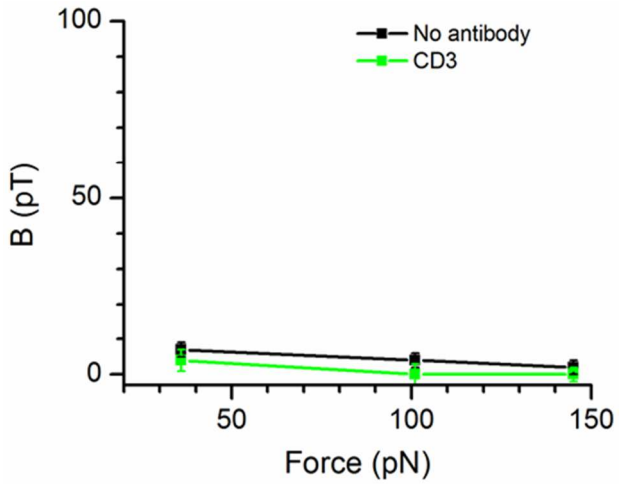

Figure S1. Magnetic signal profiles of CD4 antibody-conjugated magnetic beads binding with a non-functionalized substrate surface (black trace) and the same beads binding with a CD3 antigen-coated substrate (green trace). No signal decrease was observed in the range of 60-100

pN, which corresponds to the dissociation of the specific CD4 antibody-antigen bonds.

2. Statistics of Force Measurements

For both the CD4+ T cell surface and the CD4 antigen-functionalized substrate surface, the binding forces were measured four times, as shown in Figure S2. The average force along with its standard deviation was 75 ± 2 pN for the cell surface, and 90 ± 2 pN for the functionalized substrate surface.

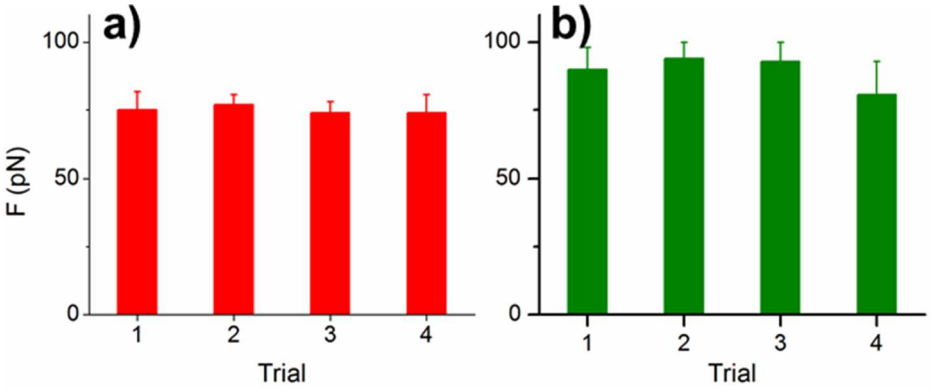

Figure S2. Binding force statistics for (a) the CD4+ T cell surface and (b) the CD4 antigen-functionalized substrate surface.

3. Image of the Cells at Reduced Density

The image in Figure S3 obtained by an optical microscope shows that most of the cells were well separated from each other.

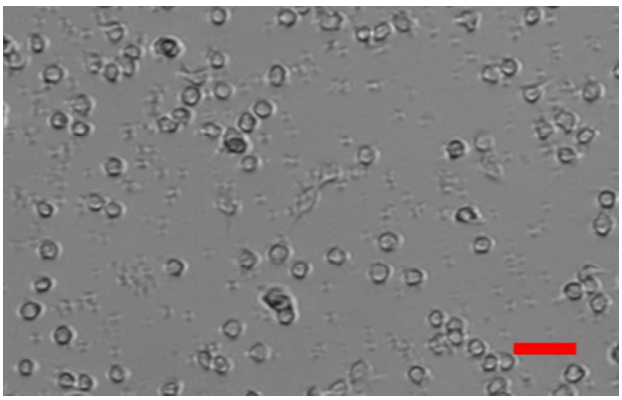

Figure S3. Optical image of the cells at a lower cell density. The total number of cells was estimated to be 2.4×10^4 for the overall sample well based on the image. Scale bar: 20 μm.

4. Binding on the Substrate Surface at Reduced Functionalization Density

To test the potential impact of functionalization density on the binding force, we significantly reduced the functionalization density by 10 times and measured the binding force (Figure S4).

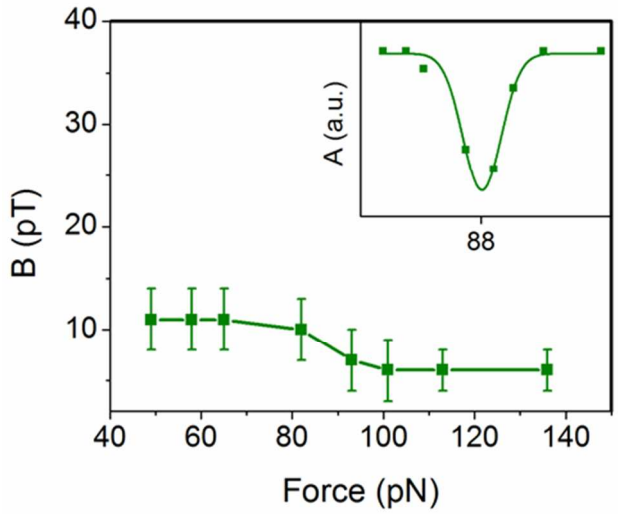

Figure S4. Magnetic signal profile of CD4 antibody-conjugated magnetic beads binding to a CD4 antigen-coated substrate surface with reduced functionalization density. The obtained binding force remained at 90 pN.

5. XPS Analysis of the Surface Antigen Density

The surface antigen density was measured by X-ray photoelectron spectroscopy (XPS). Quantification is achieved by ratioing the 1s peak of N, which indicates the presence of the antigen protein, with the 4f peak of Au in the XPS spectra (Sofia, S. J.; Premnath, V.; Merrill, E. W. *Macromolecules* **1998**, *31*, 5059-5070; Awsiuk, K.; Bernasik, A.; Kitsara, M., Budkowski, A.; Rysz, J.; Haberko, J.; Petrou, P.; Beltsios, K.; Raczkowska, J. *Colloids Surf. B*, **2010**, *80*, 63-71.). The trials 1, 2, 3, and 4 correspond to ratios of mercaptohexadecanoic acid to tetradecanethiol of 1:0, 1:4 dilution, 1:10 dilution, and 0:1, respectively. The data show that at 1:10 dilution (Trial 3), the antigen density is only 20% above the noise floor, confirming very low surface density. This result is consistent with the magnetic measurement in Figure S4.

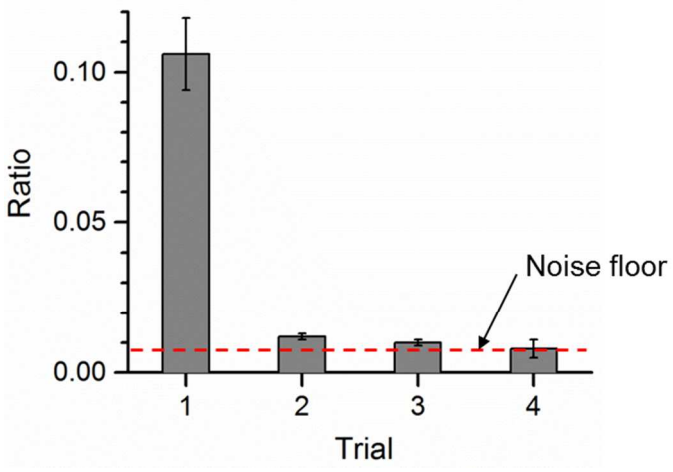

Figure S5. Quantification of the surface antigen density using XPS. Trials indicate different dilutions of the surface functionalization. The ratios were obtained by dividing the N_{1s} intensities by Au_{4f} intensities in the XPS spectra.

6. Experimental Methods

Magnetic measurement is achieved by an atomic magnetometer. The principle is shown in Figure S6. A linearly polarized laser excites the Cs atoms contained in the atomic sensor. The polarized Cs atoms will then undergo Lamar precession in the magnetic field produced by the sample, with frequency given by γB where γ is the gyromagnetic ratio (3.5 Hz/nT for Cs) and B is the magnetic field. Consequently, the polarization of the laser will be rotated. When the laser modulation frequency equals twice the Lamar frequency of the atoms, magneto-optical resonance is observed. Therefore, the resonance frequency is a direct measurement of the sample magnetic field. The sample was subject to a varying centrifugal force prior to each measurement.

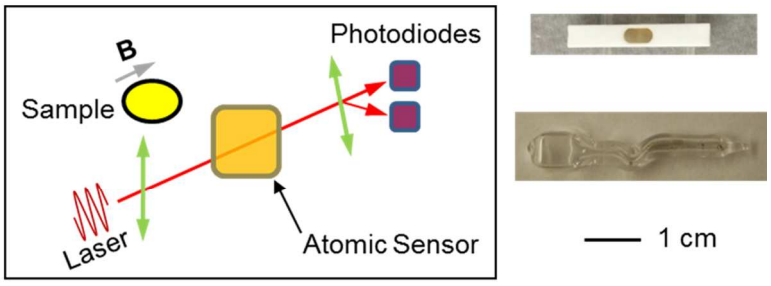

Figure S6. Schematic of the experimental method. The insets on the right show photos of the sample well (top) and the atomic sensor (bottom).

7. Information for the CD4+ T Cells

The CD4+ T cells we purchased were human leukocyte isolates and processed through negative selection (Innovative Research, Donor M7021, Product # 0001144236). During the negative selection, $CD4^+$ T cells were enriched by depleting all other necessary cells. The transport media was RPMI plasma.